American Journal of Hematology 53:133–136 (1996)

HTLV-I-Seronegative, Genome-Positive Adult T-Cell Leukemia: Report of a Case

Tetsuya Kubota, Takayuki Ikezoe, Eiji Hakoda, Takashi Sawada, Hirokuni Taguchi, and Isao Miyoshi

Department of Medicine, Kochi Medical School, Kochi, Japan

An HTLV-I-seronegative case of adult T-cell leukemia (ATL) carrying the HTLV-I genome is reported. Screening serological tests were negative and Western blot analysis revealed only a faint band for HTLV-I p24. Polymerase chain reaction (PCR) disclosed the presence of HTLV-I *gag, pol, env, pX,* and LTR sequences in the lymph node and peripheral blood. Southern blot analysis revealed a monoclonal integration of HTLV-I in the lymph node and peripheral blood. The tumor cells expressed viral antigens after short-term culture. The clinical course was consistent with ATL in that the patient exhibited hypercalcemia and abnormal lymphocytosis as well as hepatosplenomegaly and lymphadenopathy. We recommend that PCR analysis for HTLV-I be performed even in seronegative cases when ATL is clinically suspected. © 1996 Wiley-Liss, Inc.

Key words: HTLV-I, seronegative, genome-positive, ATL

INTRODUCTION

Adult T-cell leukemia (ATL) is an endemic T-cell malignancy with poor prognosis, caused by human T-cell lymphotropic virus type I (HTLV-I) [1,2]. All ATL patients are positive for anti-HTLV-I antibody [2], but rare cases of seronegative, genome-negative ATL are also reported [3]. We report here on an unusual ATL patient who reacted seronegative but proved to be genome-positive for HTLV-I.

CASE REPORT

A 72-year-old man was admitted to our hospital in November 1994 with high fever and systemic lymphadenopathy. He had no history of blood transfusion. His nephew, who was seronegative for HTLV-I, died of T-cell Ki-1 lymphoma in May 1991, which was later confirmed to be HTLV-I genome-negative by polymerase chain reaction (PCR). Physical examination on admission revealed lymphadenopathy and hepatosplenomegaly. Blood counts showed a hemoglobin of 10.9 g/dl, a platelet count of 94×10^9/l, and a white-cell count of 7.6×10^9/l with 25% of lobulated abnormal lymphocytes. Serum calcium level was 7.0 mEq/l, serum albumin 3.5 g/dl, and lactate dehydrogenase 597 IU/l. Although these data strongly suggested ATL, serological studies were negative for HTLV-I by particle agglutination (Fujirebio, Tokyo, Japan), immunofluorescence [2], and enzyme-linked immunosorbent assay (ELISA) (Eisai, Tokyo, Japan). Antibody to HTLV-I $p40^{tax}$ was also negative by ELISA. Western blot analysis repeatedly demonstrated only a faint p24 band with sera at two dilutions of 1:20 and 1:5 (Fig. 1), not fulfilling the WHO criteria of seropositivity [4]. The wife of the patient was confirmed to be HTLV-I antibody-positive by Western blot. Immunoglobulin levels were within normal range. Lymph-node biopsy and immunocytochemistry showed T-cell malignant lymphoma of the diffuse large-cell type (Fig. 2A). Surface-marker analysis demonstrated lymph node cells to be CD2+, CD3+, CD4+, CD5+, CD8−, CD25+, and HLA-DR+. Chromosome analysis of a lymph-node specimen showed an abnormal karyotype of 49, XY, +3, add(4)(q31)x2, +5, +7, add(11)(q13), add(12)(p13), add(12)(q24), add(16)(p13), add(16)(p13). Lymph-node

Received for publication December 11, 1995; accepted April 10, 1996.

Address reprint requests to Dr. Tetsuya Kubota, Department of Medicine, Kochi Medical School, Kochi 783, Japan.

cells were cultured for 3 days in RPMI 1640 containing 15% fetal calf serum and 0.5 μg/ml phytohemagglutinin. They were found to express viral antigens when reacted by immunofluorescence with an ATL patient's serum and monoclonal antibodies to HTLV-I p24 and p19 (Fig. 2B). To ascertain the association of this lymphoma with HTLV-I, we performed PCR analysis on DNA obtained from lymph-node and peripheral blood mononuclear cells using primers specific for HTLV-I *gag, pol, env, pX,* and LTR regions, as previously described [5]. We amplified proviral sequences corresponding to each of these five regions (Fig. 3). Southern blot analysis revealed a monoclonal band after *Eco*RI digestion, and three internal bands after *Pst*I digestion, in both lymph-node and peripheral blood mononuclear cells, indicating the presence of a full HTLV-I genome (Fig. 4). Several courses of CHOP therapy (cyclophosphamide, adriamycin, vincristine, and prednisolone) were given with partial remission, but the patient died of multiorgan failure 8 months after admission. Permission for autopsy was not granted.

DISCUSSION

Seropositivity for anti-HTLV-I antibody is a hallmark for making the diagnosis of ATL. In this regard, so-called antibody-negative ATL cases need to be evaluated with caution, using sensitive techniques such as PCR. Whether there exists a seronegative HTLV-I infection state is still a controversial problem [6,7]. Miyata et al. [8] addressed the issue of antibody-negative, genome-positive carriers. According to their study, anti-HTLV-I antibody titers are dependent on the virus load, and approximately 35 *gag* copies/μg of DNA are required to induce a detectable antibody response. Other disorders, in which HTLV-I sequences were detected by PCR in the absence of detectable HTLV-I antibodies, include Hodgkin's-like lymphoma [9], tropical spastic paraparesis [10], dermatopolymyositis [11], and mycosis fungoides [12,13]. The reasons for this discrepancy are not entirely clear. In the first case, the tumor-tissue DNA was negative for HTLV-I by Southern blot hybridization, suggesting that a minority of cells harbored HTLV-I. In the second case, the 5′ and 3′ LTR regions of HTLV-I could not be amplified, and this defect probably made the provirus unable to express HTLV-I antigens. In mycosis fungoides, although HTLV-I *pol* and/or *tax* sequences could be amplified in a significant proportion of cases, the proviruses were thought to contain deletions or to be defective. It is interesting to note that HTLV-I has been isolated in a transformed T-cell line from a seronegative Egyptian patient with mycosis fungoides [14].

In our patient, despite seronegativity, Southern blot and PCR analyses disclosed monoclonal integration of a full HTLV-I genome in the tumor cells. Since the wife of this patient was seropositive for HTLV-I, she may have been infected with HTLV-I from her husband by sexual contact. If so, the virus was not only infectious but also immunogenic in the spouse. From these findings, it is likely that our patient was infected with a complete HTLV-I but remained seronegative due to defective immune response or immunological tolerance to HTLV-I, as has been observed in inbred rabbits neonatally infected with HTLV-I [15]. The prevalence rate of HTLV-I seronegative, genome-positive ATL cases is unknown, since seronegative patients have not usually been evaluated by PCR. Future studies will resolve this point. Thus, patients pres-

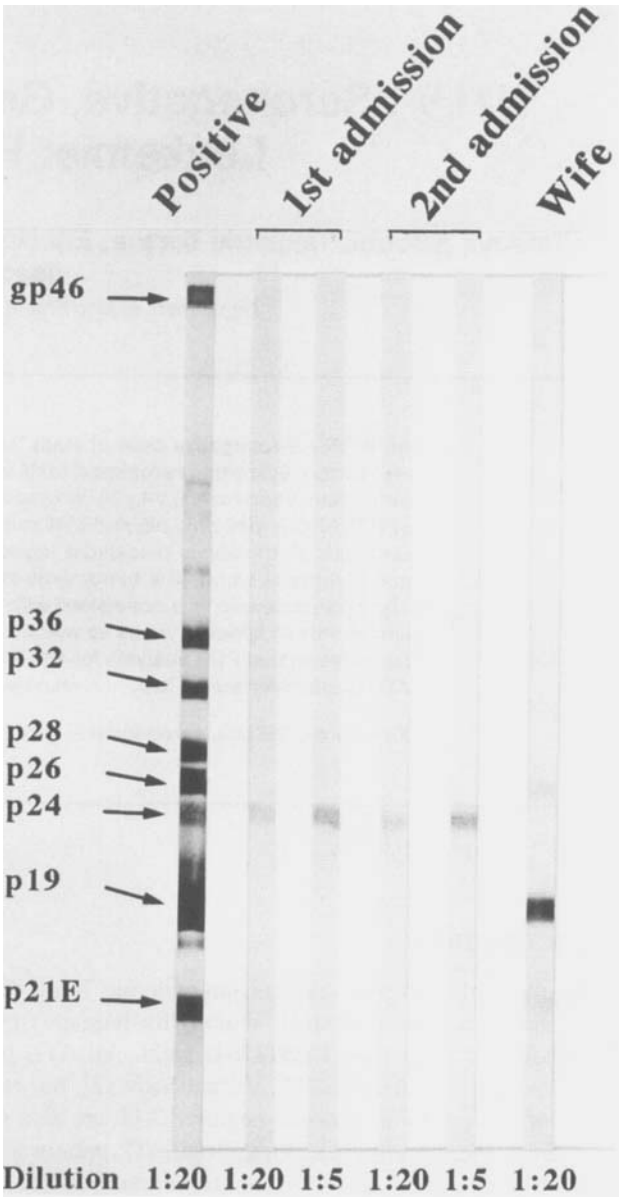

Fig. 1. Western blot of patient's sera, showing only a faint p24 band on two occasions, at 1:20 and 1:5 dilution. The patient's wife was seropositive, with reactivities with p26, p19, and p21E.

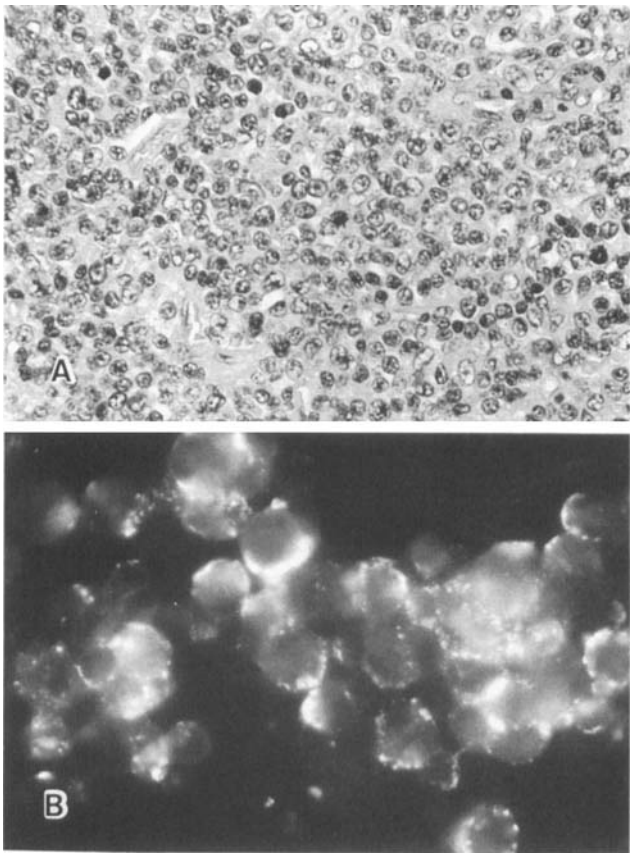

Fig. 2. A: Biopsied lymph node, showing malignant lymphoma of diffuse large-cell type (hematoxylin and eosin stain, ×450). B: Lymph-node cells after short-term culture, showing positive immunofluorescence with monoclonal antibody to HTLV-I p19 (×1,650).

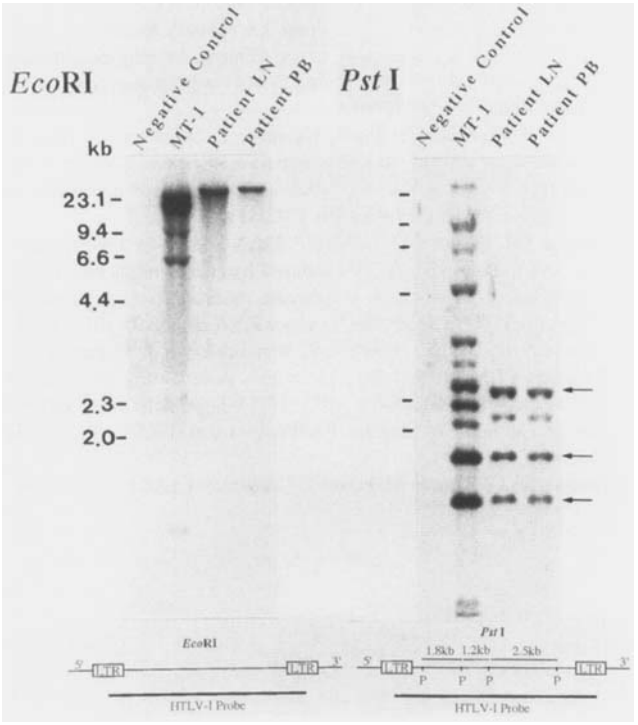

Fig. 4. Southern blot hybridization with a full-length HTLV-I probe, showing monoclonal integration of one copy of HTLV-I after *Eco*RI digestion (left) and three internal bands (arrows) after *Pst*I digestion (right) in both lymph-node and peripheral blood mononuclear cells. MT-1 shows multiple copies of HTLV-I, whereas a negative control (normal human peripheral blood mononuclear cells) shows no band.

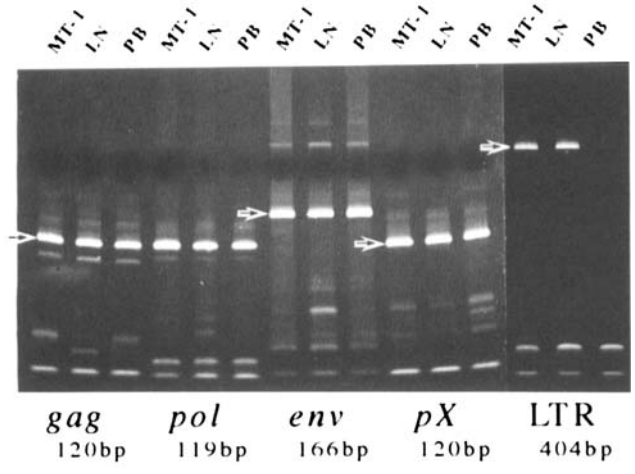

Fig. 3. PCR analysis, showing HTLV-I *gag, pol, env, pX,* and LTR sequences (arrows) in DNA from lymph-node and peripheral blood mononuclear cells. MT-1 is a positive control cell line.

enting with suspicious HTLV-I infection should be tested by PCR, even if they are seronegative by screening assays.

REFERENCES

1. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC: Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 77:7415, 1980.
2. Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita K, Shirakawa S, Miyoshi I: Adult T-cell leukemia: Antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc Natl Acad Sci USA 78:6476, 1981.
3. Shimoyama M, Kagami Y, Shimotohno K, Miwa M, Minato K, Tobinai K, Suemasu K, Sugimura T: Adult T-cell leukemia/lymphoma not associated with human T-cell leukemia virus type I. Proc Natl Acad Sci USA 83:4524, 1986.
4. World Health Organization: Acquired immunodeficiency syndrome. Proposed criteria for interpreting results from western blot assays for HIV-1, HIV-2, and HTLV-I/HTLV-II. Wkly Epidem Rec 65:281, 1990.
5. Kubota T, Morishita N, Tanaka Y, Sawada T, Miyagi T, Ohtsuki Y, Miyoshi I: Establishment of novel lymphoid cell lines dually infected with human T cell lymphotropic viruses types I and II. J Infect Dis 172:220, 1995.
6. Saito S, Ando Y, Furuki K, Kakimoto K, Tanigawa T, Moriyama I, Ichijo M, Nakamura M, Ohtani K, Sugamura K: Detection of

HTLV-I genome in seronegative infants born to HTLV-I seropositive mothers by polymerase chain reaction. Jpn J Cancer Res 80:808, 1989.
7. Pate EJ, Wiktor SZ, Shaw GM, Champegnie E, Murphy EL, Blattner WA: Lack of viral latency of human T-cell lymphotropic virus type I. N Engl J Med 325:284, 1991.
8. Miyata H, Kamahora T, Iha S, Katamine S, Miyamoto T, Hino S: Dependency of antibody titer on provirus load in human T lymphotropic virus type I carriers: An interpretation for the minor population of seronegative carriers. J Infect Dis 171:1455, 1995.
9. Duggan DB, Ehrlich GD, Davey FP, Kwok S, Sninsky J, Goldberg J, Baltrucki L, Poiesz BJ: HTLV-I-induced lymphoma mimicking Hodgkin's disease. Diagnosis by polymerase chain reaction amplification of specific HTLV-I sequences in tumor DNA. Blood 71:1027, 1988.
10. Daenke S, Parker CE, Niewiesk S, Newsom-Davis J, Nightingale S, Bangham CRM: Spastic paraparesis in a patient carrying defective human T cell leukemia virus type I (HTLV-I) provirus sequences but lacking a humoral or cytotoxic T cell response to HTLV-I. J Infect Dis 169:941, 1994.
11. Desgranges C, D'Incan M, Friard S, Caubarrere I, Couderc LJ: Detection of human T cell lymphotropic virus type I (HTLV-I)-DNA by polymerase chain reaction in an HTLV-I-seronegative patient with dermatopolymyositis. J Virus Dis 1:6, 1993.
12. Manca N, Piacentini E, Gelmi M, Calzavara P, Manganoni MA, Glukhov A, Gargiulo F, Francesco MD, Pirali F, Panfilis GD, Turano A: Persistence of human T cell lymphotropic virus type 1 (HTLV-1) sequences in peripheral blood mononuclear cells from patients with mycosis fungoides. J Exp Med 180:1973, 1994.
13. Pancake BA, Zucker-Franklin D, Coutavas EE: The cutaneous T cell lymphoma, mycosis fungoides, is a human T cell lymphotropic virus-associated disease. J Clin Invest 95:547, 1995.
14. El-Farrash MA, Salem HA, Kuroda MJ, Morizono K, Kannagi M, Harada S: Isolation of human T-cell leukemia virus type I from a transformed T-cell line derived spontaneously from lymphocytes of a seronegative Egyptian patient with mycosis fungoides. Blood 86:1842, 1995.
15. Seto A, Kawanishi M, Matsuda S, Ogawa K: Seronegative virus carriers in the infection of rabbits with human T lymphotropic virus type I. J Exp Med 168:2409, 1988.